The antidepressant sertraline provides a novel host directed therapy module for augmenting TB therapy

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Running TItle: The antidepressant sertraline augments antimycobacterial therapy

ABSTRACT:

A prolonged therapy regimen, primarily responsible for development of drug resistance by *Mycobacterium tuberculosis* (Mtb), the causative agent of human TB, obligates any new regimen to not only reduce treatment duration but also escape pathogen resistance mechanisms. With the aim of harnessing the host response in providing additional support to existing regimens (host directed therapy- HDT), we established the ability of a well-tolerated anti-depressant (sertraline - SRT) to modulate the pro-pathogenic type I IFN response of macrophages to Mtb infection. More importantly, while SRT alone could only arrest bacterial growth, it could effectively escalate the bactericidal activities of Isoniazid (H) and Rifampicin (R) in macrophages. This strengthening of antibiotic potencies by SRT was more evident in conditions of ineffective control by these frontline TB drugs: against HR tolerant strains or dormant Mtb. SRT, could significantly combine with standard TB drugs to enhance early pathogen clearance from tissues of mice infected with either drug sensitive/ tolerant strains of Mtb. Further, we demonstrate an enhanced protection of the highly susceptible C3HeB/FeJ mice in an acute model of TB infection with the combination therapy signifying the use of SRT

as a potent adjunct to standard TB therapeutic regimens against bacterial populations of diverse physiology. This study advocates a novel host directed adjunct therapy regimen for TB with an FDA approved and well tolerated anti-depressant to achieve quicker and better control of infection.

Abbreviations: IFN- Interferon, SRT- sertraline hydrochloride, Mtb- *Mycobacterium tuberculosis,* HDT- host directed therapy

Introduction:

The current TB therapy regimen ranging between 6 months for pulmonary and 1-2 years for extrapulmonary infections, is often associated with severe drug induced toxicity in patients. Moreover, its failure to completely eradicate the pathogen from the host forms an ideal platform for the emergence of drug resistant strains [1,2]. It is not surprising that these strains have emerged at an alarming rate in the population and are imposing serious impediments to TB control programs globally [3,4]. Introduction of newer modalities like Host directed therapies (HDT) with the potential to reduce duration of therapy and not be affected by pathogen resistance mechanisms offer significant advantages in this scenario [5,6]. Several strategies for HDT with diverse modes of action- boosting immune response [7,8],[9], targeting virulence mechanisms [10-12], augmenting host metabolism [13] and host nutrition [14] have been identified in recent times. Effective molecular entities like antibodies [15,16], cytokines [17,18], cell based therapies [19], drugs used for other human non- infectious diseases and recombinant proteins [20] have been tested against bacterial infections like *Streptococcus pneumoniae*[16], *Bordetella pertussis* [21,22], *Helicobacter pylori* [23] and against viral infections like HIV [24], CMV [25], Hepatitis C [18], influenza [26] and Ebola viruses [27].

Mtb infection invokes several mechanisms of pathogen clearance in host cells like induction of pro-inflammatory response, metabolic stress, phago-lysosomal lysis programs, apoptosis/ autophagic mechanisms [28-30]. Co-evolution with humans has helped Mtb adapt and survive these host derived stresses through complex and intricate interactions to facilitate optimal

53 infection [31-38]. Extensive efforts have led to the development of therapeutic strategies countering
54 the pathogen mediated subversion of cellular clearance mechanisms [14,39-43].
55 A prominent response of Mtb infected macrophages is the early and robust induction of type I
56 IFN signaling that is associated with a detrimental effect on host survival [44-47]. We sought to
57 offset this response in macrophages and evaluate the effect on the infection dynamics. A
58 previous study identified several antipsychotic molecules as potent antagonists of Type I IFN
59 response to polyI:C [48]. We hypothesized that this property of antidepressants as TI IFN
60 antagonists might help stunt this macrophage response. In line with this hypothesis, we
61 demonstrate that the FDA approved antidepressant, sertraline, previously known to inhibit the
62 formation of the pIRF3- activation complex formation to effectively inhibit infection induced IFN.
63 We found this inhibition manifesting as a growth arrest of Mtb in macrophages. Interestingly,
64 SRT could augment mycobacterial killing in the presence of INH (H) and rifampicin (R), two of
65 the frontline TB drugs in macrophages by effectively lowering the concentration of antibiotics
66 required to achieve clearance. Remarkably, the combination proved effective even against
67 dormant bacilli or antibiotic tolerant Mtb strains. Addition of SRT to TB drugs- HR or HRZE (HR+
68 pyrazinamide, ethambutol) significantly protected infected mice from TB related pathology both
69 by enhancing bacterial clearance and host survival, implying on the usefulness of this
70 combination therapy in both the intensive (bactericidal) and continuation (sterilizing) phases of
71 anti TB therapy (ATT). Taken together, we report a novel adjunct TB therapy module by
72 repurposing the FDA approved, prescription antidepressant -sertraline.
73 **Results:**
74 Macrophages respond to mycobacterial infection by elaborating an array of signaling cascades
75 and effector functions [49-51] . Several groups have identified type I IFN signaling as an active and
76 dominant response of THP1 macrophages to Mtb infection [44,46,52-54]. We questioned the benefit
77 to Mtb in actively inducing this response and hypothesized that suppressing this response in
78 cells would alter macrophage infection dynamics. Our previous work indicates a role for nucleic

79 acid sensing in the Mtb induced Type I IFN response [47]. As an initial step in this direction, we
80 chose previously identified inhibitors of RNA induced Type I IFN response that were also FDA
81 approved drugs. We used sertraline, a TLR3 signaling antagonist for the ability to modulate Mtb
82 induced type I IFN response in macrophages. We observed a dose dependent reduction in Mtb
83 induced type I IFN response of macrophages on treatment with SRT (Fig.1A). While 1μM was
84 minimally inhibitory, 5μM and 10μM of SRT inhibited the response by 35 and 43% respectively.
85 At a dose of 20μM, SRT significantly reduced the response to 1/5th of the untreated values (Fig.
86 1A). While naïve macrophages were permissive for Mtb growth to 10 folds of input by day 5,
87 20μM SRT treated macrophages prevented mycobacterial growth (Fig.1B). In contrast 20μM
88 SRT alone led to only 2-fold reduction in Mtb growth *in vitro* (data not shown). The observed
89 bacterial stasis in SRT treated macrophages prompted us to analyze the effect of this treatment
90 in conjunction with frontline antibiotics. To test this, we enumerated Mtb growth in macrophages
91 following treatment with 200ng/ml INH and 1000ng/ml Rifampicin (HR) alone or in combination
92 with 20μM SRT (Fig. 1C). HR treated macrophages could restrict bacterial numbers by ~62 fold
93 by day 3 and ~92 fold by day 5. An additional 3-fold increase in bacterial killing was observed
94 within day 3 by addition of SRT to the antibiotics (Fig. 1C). Given the effective control of bacteria
95 by HR, we reasoned that any additional effect of SRT would be better visible with lower
96 concentrations of antibiotics. The effective killing capacity decreased exponentially as the
97 concentrations were lowered by 10 folds, nearly allowing bacterial growth (Fig. 1C). At 20μM
98 SRT with 0.1X dose of HR, the bacterial load decreased by 2 logs, attaining similar levels as HR
99 alone at 1X concentration (Fig. 1C). Even at a concentration of 0.04X, where HR did not show
100 bactericidal activities, addition of SRT reduced bacterial numbers by 10-20 folds by day 5,
101 indicating substantial boosting of antibiotic efficacy in this group (Fig. 1D).
102 To determine if the effect of SRT was preserved in primary human macrophages, we repeated
103 these experiment in monocyte derived macrophages from 7 healthy individuals (Fig. 1E).
104 Although Mtb growth varied in the different individuals, SRT alone was not effective in altering

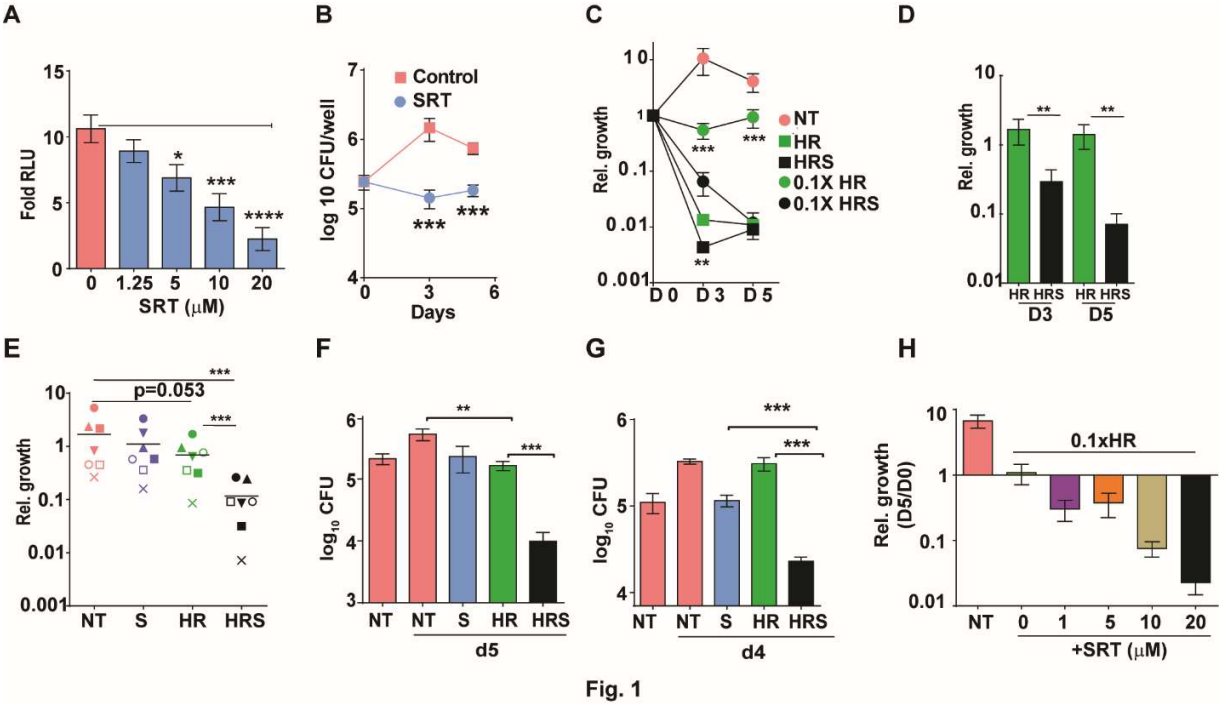
105 the growth across MDMs from different donors. 0.1X HR exhibited variability in control across
106 individuals. However, SRT resulted in strong synergistic anti-bacterial activity resulting in 10-15
107 folds lower bacterial, numbers arguing for a universal adjunct activity of SRT to frontline TB
108 drugs.

109 The ability of Mtb to enter into a non-replicating dormant state associated with tolerance to
110 frontline drugs is an important limitation of the current treatment regimen. The ability of SRT to
111 augment Mtb killing warranted a testing of its efficacy in conditions of antibiotic failure. The
112 recent model of vitamin C induced dormancy in Mtb replicates this scenario with a complete loss
113 of isoniazid and rifampicin efficacy in bacterial control [55]. To test the effectiveness of the adjunct
114 therapy in this model, we incubated infected THP1 macrophages with VitC to induce dormancy
115 in bacteria [55]. SRT was able to induce bacterial stasis in this model, while HR was ineffective at
116 controlling bacterial growth. However, HR along with SRT led to 10-12 fold reduction in bacterial
117 numbers within 4 days of treatment indicating its activity against dormant Mtb (Fig. 1F).

118 More recently, the inability of drugs to distribute equally amongst the spectrum of
119 granulomatous lesions was recognized as an important factor in promoting bacterial tolerance
120 and resistance with the lipid loaded necrotic lesions forming a formidable barrier for entry of
121 frontline TB drugs *in vivo* [56,57]. *In vitro* studies with THP1 macrophages to increase intracellular
122 lipid content (treatment with oleic acid) mimic conditions of foamy macrophages in advanced
123 necrotic granuloma [58], [59]. As an indirect correlate of efficacy of the combination in these
124 conditions, we tested antibiotic killing in THP1 cells pre- treated with oleic acid. Again, HR was
125 minimally antibacterial in macrophages treated with oleic acid (Fig. 1G). In contrast, the
126 combination led to more than 10 fold reduction in bacterial loads as compared to untreated and
127 HR treated macrophages.

128 Furthermore, we found that SRT at concentrations as low as 1 μM could potentiate antibiotic
129 mediated growth restriction in macrophages (Fig. 1H). In order to better understand the
130 mechanism of SRT mediated control of bacterial growth, we first tested if SRT was also potent

131 in supplementing the activity of HR in *in vitro* culture. Even a 6-fold higher concentration of SRT
132 failed to impact Mtb growth either alone or in combination with 3 concentrations of HR under *in*
133 *vitro* growth conditions (Supplementary Figure 1). These results hinted at an indirect effect of
134 SRT in enhancing the ability of macrophages to control bacteria rather than a direct benefit to
135 the microbicidal properties of the antibiotics.



136

137 **Fig. 1: Sertraline inhibits Mtb induced Type I IFN response and restricts intra-macrophage**
138 **Mtb growth**

139 A) IRF dependent luciferase activity in THP1 Dual macrophages following infection with Mtb at a
140 MOI of 5. Cells were left untreated or treated with increasing concentrations of SRT for 24h in
141 culture and the luminescence in culture supernatants was measured and is represented as mean ±
142 SEM from 2 independent experiments with triplicate wells each. B) Intracellular bacterial numbers
143 in macrophages infected with Mtb for 6h at MOI of 5 and then left untreated (NT) or treated with
144 20μM SRT. Intracellular bacterial numbers at the indicated time points are represented as average
145 $\log_{10}$ CFU ± SEM from 2 independent experiments with triplicate wells each. (unpaired t-test,
146 **p<0.01, ***p<0.001).

147 B-H) Intracellular bacterial numbers in THP1 Dual macrophages following infection with Mtb at
148 MOI5 for 6h and then either left untreated (NT- red) or treated with, Sertraline (SRT/ S-blue), (HR -
149 200ng/ml INH and 1000ng/ml Rif- green) or a combination of all three (HRS- black). B- comparison
150 of bacterial growth in untreated or SRT treated macrophages, C) growth of Mtb in cells not treated
151 or treated HR at 1X and ten- fold lower (0.1X) concentrations with and without SRT. D) Intracellular
152 bacterial survival in macrophages treated with 0.04X HR+20μM SRT, represented as growth
153 relative to treatment without SRT. E) Mtb growth in primary human M1- differentiated MDMs from
154 PBMC of seven individuals is represented as CFU counts relative to initial CFU in untreated
155 samples. Each symbol represents one individual, colors depict the treatment groups as before. F,
156 G) Growth in macrophages treated with VitC (F) for 24h post infection or with 200μM Oleic acid for
157 48h prior to infection (G). H- macrophages treated with 0.1X HR with different concentrations of
158 SRT. Bacterial numbers were enumerated and is represented as average $\log_{10}$ CFU ± SEM from 2-
159 3 independent experiments with triplicate wells each. Except for E, paired t-test comparing ratios,
160 other datasets were compared with unpaired t-test; **p<0.01, ***p<0.001).

161 To test if SRT induced mycobacterial stasis in macrophages was due to inhibition of type I IFN
162 expression, we analyzed growth of Mtb in RAW264.7 macrophages deficient in this signaling
163 component. In contrast to the growth in WT macrophages, Mtb was significantly restricted in
164 STING$^{-/-}$ and TRIF$^{-/-}$ macrophages to the initial input levels even after 5 days of culture (Fig. 2A).
165 Again, the persistent levels of bacterial CFUs without a significant decrease by 5 days mirrored
166 the situation of bacterial stasis observed in SRT treated macrophages. Evidence for interferon
167 signaling interference by SRT was also apparent by the 4-fold reduction of IP10 expression
168 levels in Mtb infected macrophages treated with HRS as compared to HR alone after 18h of
169 treatment (Fig. 2B). Further confirmation was achieved by treating macrophages with a more
170 potent inhibitor of type I IFN signaling- BX795, that completely abrogated Mtb induced type I IFN
171 at a very low concentrations of 1.2 μM (Fig. 2C). While BX795 alone was only capable of
172 bacteriostasis in macrophages (Fig. 2D), its addition to ineffective concentrations of HR
173 enhanced antibiotic mediated bacterial killing by 4-5 folds by 3 days of infection (Fig. 2E). A

174 recent study by Teles et.al, 2013 suggested the active suppression of IFNγ mediated activation
175 of macrophage mediated killing of mycobacteria as one of the potent pro-pathogenic effects of
176 type I IFN in mycobacterial infections [60]. We hypothesized that if SRT was efficient in controlling
177 Mtb induced macrophage type I IFN induction the effects of IFNγ mediated activation would be
178 more efficient in controlling infection. Indeed, we found 2-fold enhancement in IFNγ mediated
179 control in SRT treated macrophages (Fig. 2F). To further probe into the molecular mechanism of
180 SRT mediated IFN suppression, we analyzed the pro-inflammatory response of Mtb infected
181 macrophages treated with SRT (Fig 2G-J). Gene expression profiles at 18h of treatment
182 showed reduction in the expression of TNF and IL1β with SRT treatment. In comparison to HR
183 treated cells, TNF expression was reduced only slightly in the HRS treated macrophages (Fig.
184 2G). However, the levels of secreted TNF was significantly altered by SRT treatment; in SRT
185 alone or HRS treated macrophages, negligible levels of TNF was observed in the cell
186 supernatants, contrasting with the high levels of TNF (between 500pg- 1ng/ml) in the case of
187 Mtb infected cells with or without treatment with HR (Fig. 2H).
188 Surprisingly, while expression levels of IL1β were 2-3 folds lower in HRS treated macrophages
189 by 18h (Fig. 2I), the amount of secreted cytokine was significantly elevated (> 2-3 folds) in
190 macrophages treated with SRT/ HRS from 18h until 66h (Fig. 2J). These data strongly indicated
191 potential activation of the host cell inflammasome by SRT. In fact, with the studies supporting
192 the antagonism between type I IFN and inflammasome activation, we tested the efficacy of SRT
193 to potentiate antibiotic mediated killing in the presence of inflammasome inhibitors. Again, while
194 SRT enhanced the ability of HR to control Mtb in macrophages, pretreatment of cells with the

195 inflammasome inhibitor, isoliquiritigenin (I), completely nullified the boosting effect of SRT on
196 antibiotic efficacy (Fig. 2K), suggesting that the type I IFN modulating effect of SRT was
197 mediated in part by inflammasome activation.

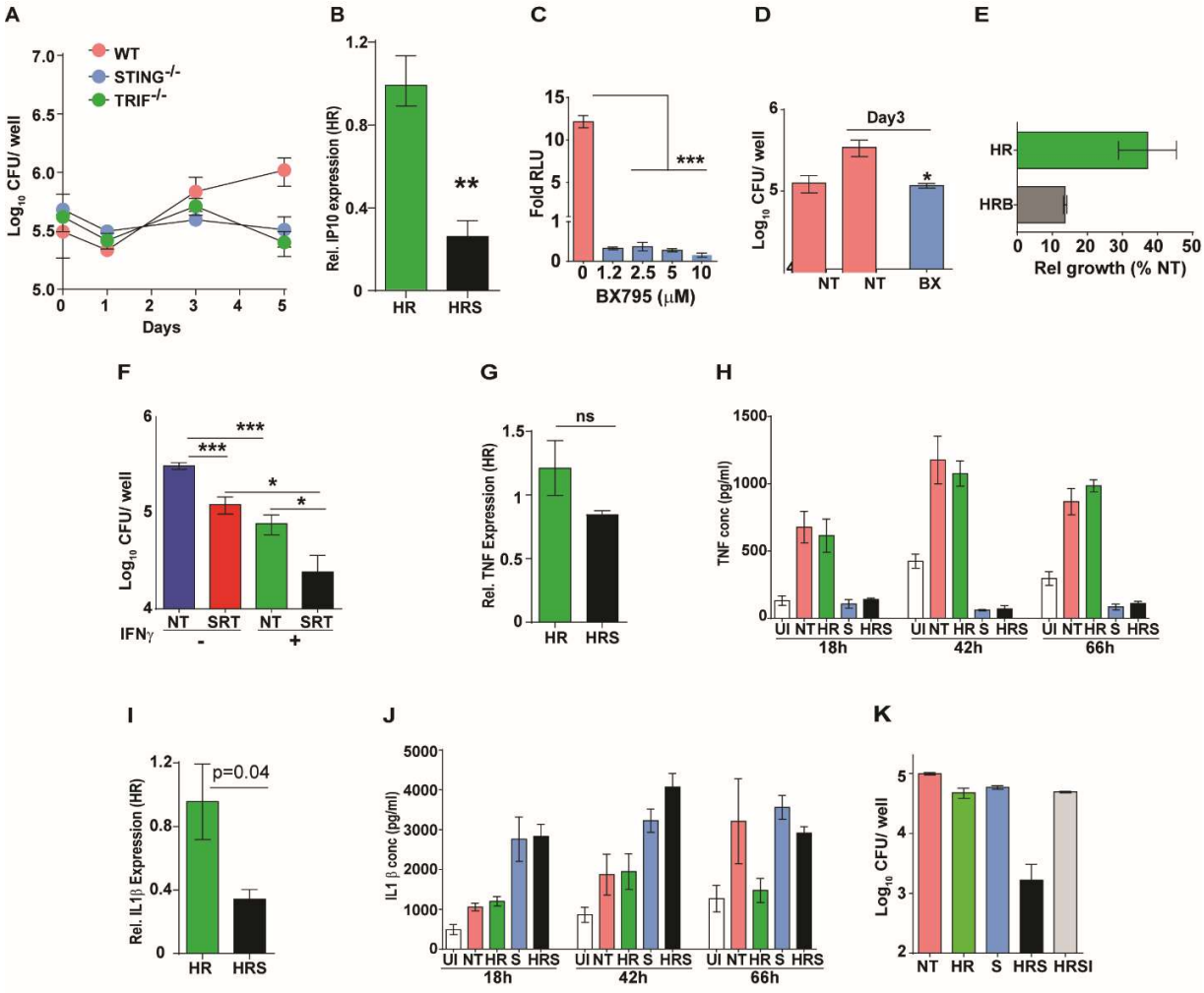


Fig. 2

198

199 **Fig. 2: Augmentation property of SRT is due to its ability to inhibit IFN signaling.**

200 A, D-F) Growth of Mtb in macrophages following infection at MOI5 for 6h and left untreated (NT) or
201 treated with HR at 0.01X MIC with and without SRT. A- Intracellular bacterial growth in WT, STING-
202 /- or TRIF-/- deficient RAW264.7 macrophages represented as mean ± SD log_{10} CFU/ well of

203 triplicate wells of a representative experiment of two, B) Expression of IP10 in macrophages
204 infected with Mtb for 6h and then treated with HR alone or in combination with SRT for 18h. The
205 values represented are relative fold expression levels in HRS w.r.t macrophages treated with HR
206 alone ± SEM from two independent experiment with duplicate wells each. C) IRF dependent
207 luciferase activity in THP1 dual macrophages 24h after treatment with varying doses of BX795
208 along with infection with Mtb at MOI of 5. D) Bacterial growth in macrophages left untreated or
209 treated with 10μm BX795. E) Relative growth of Mtb in macrophages treated with HR and
210 HR+Bx795 (HRB) for 3 days. The percentage relative growth of intracellular bacterial numbers in
211 HR or HRB groups with respect to untreated samples. F) Growth of Mtb in macrophages pre-
212 treated with IFNγ for 16h, infected with Mtb for 6h and then left untreated or treated with SRT for 3
213 days. G-J) Relative transcript abundance and secreted TNF (G and H respectively) and IL1β (I and
214 J respectively) in macrophages infected with Mtb at MOI 5 for 6h and then treated with HR alone or
215 in combination with SRT. Data in G and I depict fold expression relative to HR alone as mean ±
216 SEM from two independent experiments with duplicate wells each at 18h post treatment. Data in H
217 and J depict secreted cytokines at indicated time points post treatment as mean ± SD for n=3. K)
218 Growth of Mtb in macrophages infected with Mtb for 6h and then left untreated or treated with HR,
219 SRT, HRS, HRS+ isoliquiritigenin (I) for 5 days. Data is represented as mean + SEM for 2-3
220 independent experiments containing triplicate wells per assay and analysed by (unpaired t-test,
221 ns=not significant, **p<0.01, ***p<0.001).

222 With potent efficacy boosting properties of SRT in macrophages, we tested the *in vivo* efficacy
223 of the combination regimen in an extremely susceptible model of TB infection. We reasoned that
224 the survival of TB infected C3HeB/FeJ mice would provide an optimal platform for a fast readout
225 of comparative drug efficacies of standard TB drugs and the adjunct regimen with SRT. Given
226 the excellent ability of infection control by INH and Rifampicin in this model, it was necessary to
227 test the adjunct effect of SRT in conditions of minimal/ no advantage imparted by the frontline
228 drug combination alone *i.e.* lower doses -C2 (0.1X) and C3 (0.01X) in addition to the standard
229 dose C1 of HR *ad libitum* in drinking water (Fig. 3A). Aerosol delivery of Mtb at a high dose of

230 ~500 cfu/animal, resulted in precipitous disease with rapid killing of untreated (NT) animals by
231 day 31 of infection (Fig. 3B). To facilitate disease progression prior to treatment initiation,
232 animals infected with Mtb were left untreated for 2 weeks. SRT alone was effective in delaying
233 the disease progression in animals as the mean survival time (MST) increased from 31 days for
234 untreated animals to 38 days. HR at the lowest concentration (C3), similar to SRT, delayed
235 animal mortality with a mean survival time of 41 days. Given the higher susceptibilities of female
236 mice to infection observed in our study, we decided to differentially tally the gender-based
237 effects of SRT treatment in these animals. The combination of HRC3 and SRT, nearly doubled
238 the MST of both female and male mice to 78 days and 100 days from 41 and 45 days
239 respectively (Fig. 3C). This benefit of SRT was similar to that observed for a 10-fold higher
240 concentration of HR alone (MST of 85 for HRC2) (Fig. 3B). The lower efficacy of antibiotics
241 alone was also reflected in the gross pathology of lungs by 30 days; infected animals showed
242 extensive progressive granulomas despite treatment with antibiotics while a significant
243 amelioration of pathology was observed for the group of animals on combination regimen (Fig.
244 3D).
245 We wanted to explore temporal benefits of the combination regimen after withdrawal of a limited
246 term treatment (Fig. 3E). In this model, 100% of animals survived after treatment with HR(C1) at
247 16 weeks post infection (Fig. 3F). A 7-week *ad libitum* treatment with the 10-fold lower dose of
248 HR (HRC2) (Fig. 3F) significantly decreased the MST of animals to 85 days with 100% mortality
249 by 16 weeks. In contrast, 40% of HRC2S treated animals, survived with a MST of 112 days for
250 the group (Fig. 3F, G). All animals with the highest dose of HR (HRC1) either alone or with SRT
251 survived the infection. Despite, the significant heterogeneity of treatment response in male and
252 female mice, with a relatively lower response as evidenced by the greater number of lesions in
253 lungs of male animals, a co-operative effect of SRT inclusion was evident as a significant
254 improvement in TB associated lung pathology (Fig. 3H). Small macroscopic lesions were
255 observed in lungs of 60% of the female mice treated with HRC1 that showed as multiple, well

defined granuloma in the H&E stained sections by the 16th week post infection. In contrast, mice treated with SRT and HRC1 showed negligible involvement of the lung tissue in granulomatous cellular accumulation. Even in tissues of male mice, animals receiving the adjunct therapy showed fewer macroscopic and significantly lower numbers of microscopic granuloma in lung sections in comparison to animals treated with the antibiotics alone.

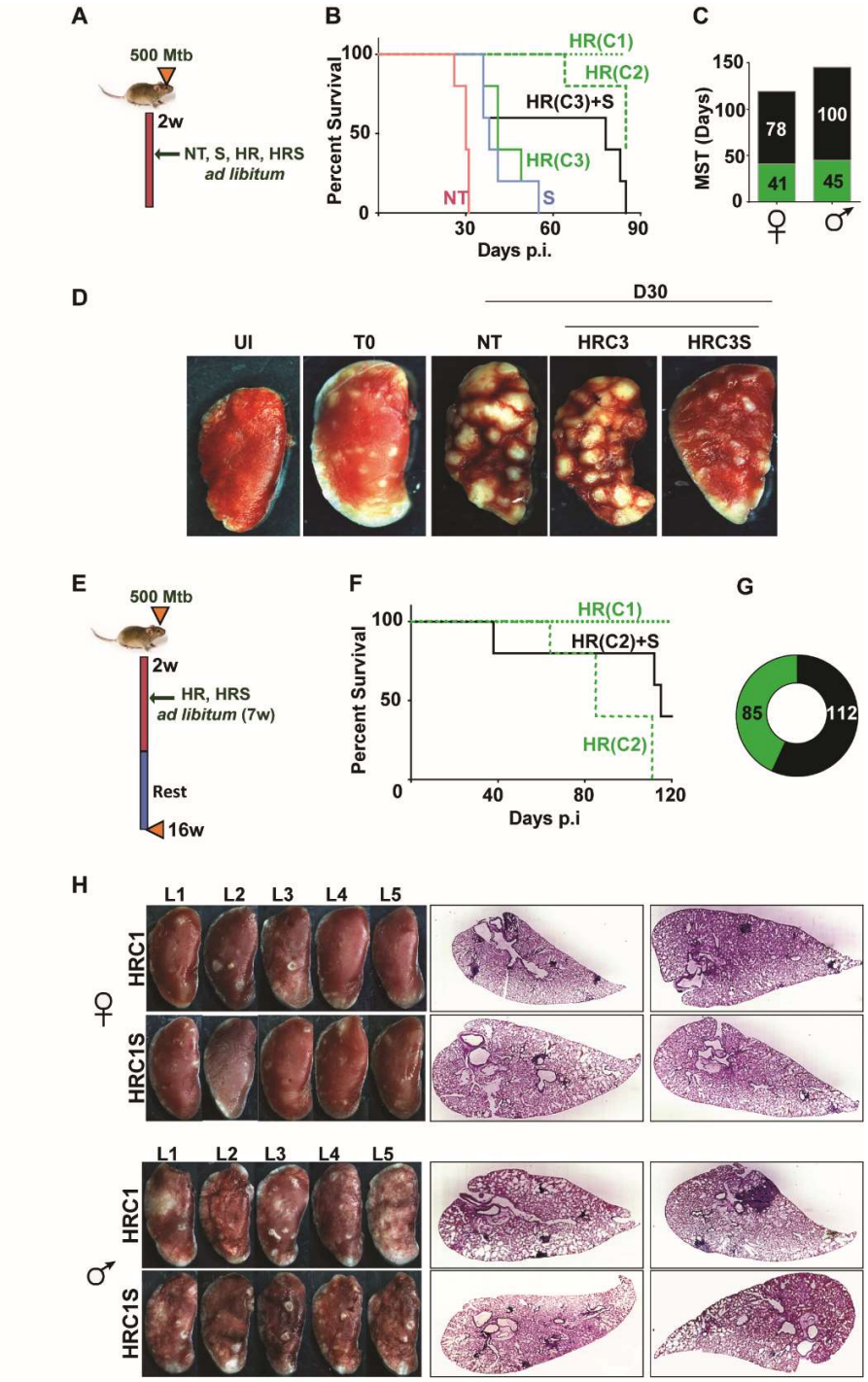


Fig. 3

282 **Fig. 3: Adjunct SRT improves host survival in a susceptible mouse model of infection.**

283 A) Schematic of Mtb infection and drug treatment in C3HeB/FeJ female mice. B) Survival curve of
284 Mtb infected female C3HeB/FeJ mice treated with different concentrations of H and R (HRC1- 1X:
285 H-100μg/ml, R-40μg/ml, HRC2-0.1X, HRC3-0.01X) alone or along with SRT (10μg/ml). C)
286 Median survival time of different treatment groups of mice. D) Gross tissue morphology of lungs
287 of uninfected animals (UI) and indicated groups at 30 days post infection with Mtb. E) Schematic
288 of infection and antibiotic treatment in C3Heb/FeJ with HRC2 and HRC1. F-G) Survival (F) and
289 MST (G) of C3Heb/FeJ mice treated with HRC2 or HRC2S. H) Gross lung morphology at the end
290 of 16 weeks and histochemical sections of lungs with H&E staining of C3HeB/FeJ mice either
291 treated with HRC1 or in combination with SRT.

292 TB treatment in the intensive phase involves the use of 4 frontline TB drugs- HRZE for a period
293 of two months and HR for an additional 4 months. Further to test the efficacy of SRT in
294 combination with HRZE in an acute model of disease, Mtb infected C3HeB/FeJ mice were
295 treated either with the established dose of HRZE or in combination with SRT (Fig. 4A) and
296 evaluated TB associated pathology of the lungs at 16 weeks of infection. Male (Fig. 4B) and
297 female (Fig. 4C) animals treated with the 4 drugs for 7 weeks harbored 10^5-10^6 bacteria in their
298 lungs, respectively. Addition of SRT appreciably improved drug efficacy, reducing the lung
299 bacterial loads by a further 5-7 folds (Fig. 4B and C).

300 The drugs efficiently lowered tissue pathology as evidenced by the macroscopic lesions seen in
301 the lungs of infected mice (Fig. 4D). While both female and male mice showed small
302 macroscopic lesions in lungs on treatment with HRZE, despite the heterogeneity between the
303 genders, the combination of SRT and HRZE sufficiently decreased the extent of tissue
304 involvement in TB associated pathology. This difference was more evident in tissue sections,
305 wherein animals treated with HRZES were devoid of granulomatous infiltrates in contrast to the
306 HRZE treated animals which had significantly higher numbers of granulomas in the lungs (Fig.
307 4D).

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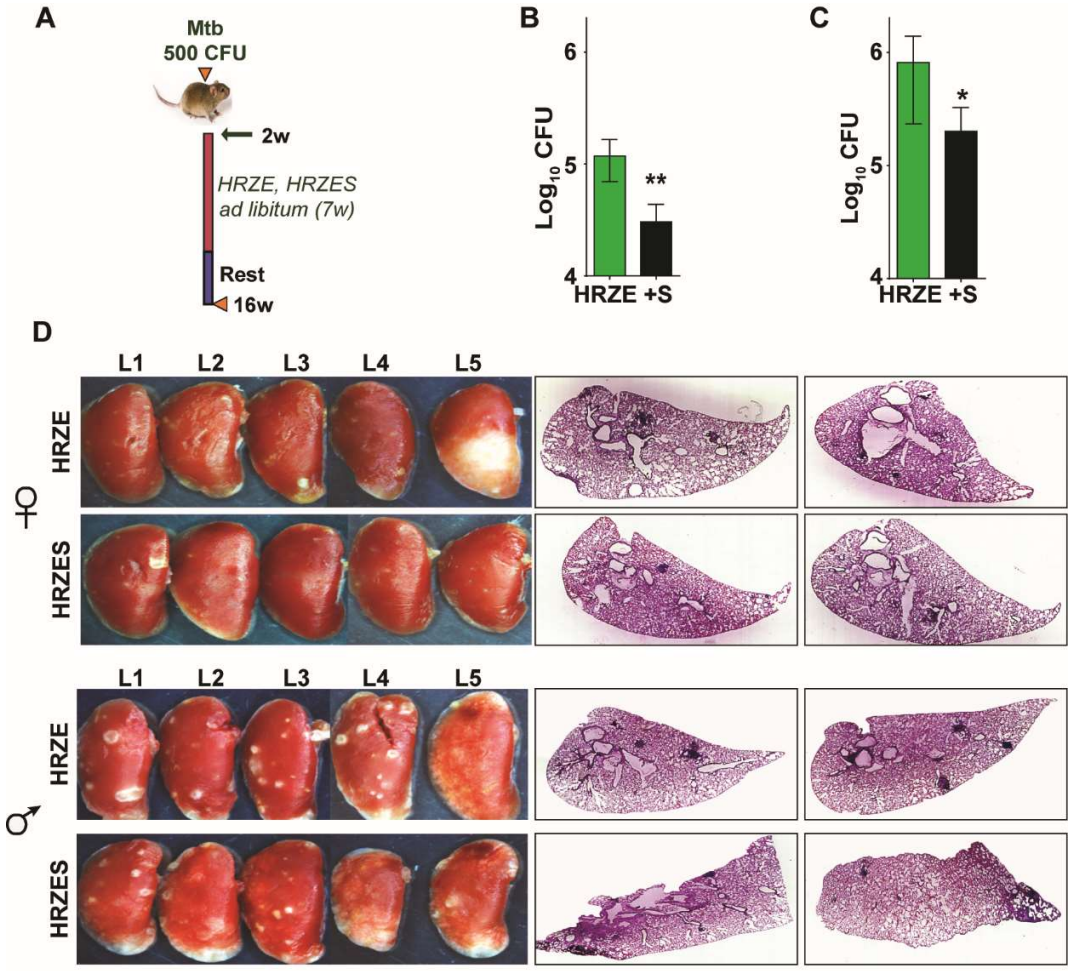

Fig. 4

Fig. 4: *In vivo* potentiation of SRT mediated antimycobacterial activity

(A) Schematic of infected C3Heb/FeJ mice and treated with HRZE (HRC1- 1x: H-100μg/ml, R-40μg/ml, Z-150μg/ml, E-100μg/ml or in combination with SRT (10μg/ml). Animals were euthanized at the end of 16 weeks and extent of infection was determined by estimating bacterial numbers (CFU) in lungs of female (B) and male (C) mice. D) Gross lung morphology and histochemical sections of lungs with H&E staining of C3HeB/FeJ mice infected with Mtb and treated either with HRZE or in combination with SRT. (unpaired t-test,*$p<0.05$, **$p<0.01$).

To evaluate the adjunct regimen for early bacterial clearance rates, we infected C57BL/6 mice with 500 CFU of Mtb and enumerated bacterial burdens at 1- and 3-weeks post treatment according to the schedule shown in Fig. 5A. Bacterial numbers in the lungs reached ~10^7 CFU

337 by 4 weeks of infection (day 0 of treatment) and remained steady over the 6-week period in
338 untreated animals (Fig. 5B). While treatment with HRZE was efficient in steadily reducing these
339 numbers by ~100 folds, addition of SRT to the regimen significantly enhanced control by a
340 further 2-3 folds. Moreover, the adjunct regimen was efficient in controlling dissemination of
341 infection into spleens of infected mice (Fig. 5C). Although HRZE reduced splenic bacterial
342 numbers significantly by 6-7 folds, HRZES was more potent reducing bacterial numbers further
343 by ~20 folds by 21 days of treatment. A similar degree of enhanced bacterial control (4-6 folds
344 lesser bacteria) was observed in lungs and spleens of Balb/c mice treated with SRT as an
345 adjunct to conventional 4 drug-therapy (Fig. 5D). Untreated animal lungs showed a gradual
346 consolidation of the tissue with increasing amounts of granulomatous cellular infiltration by 6
347 weeks of infection. Treatment with HRZE was efficient in reducing this infiltration significantly by
348 the 6th week of infection with nearly $1/6^{th}$ of the tissue showing signs of cellular infiltration. Lungs
349 of mice receiving the combination showed better resolution of granulomas with significantly
350 smaller regions of cellular collection by 3 weeks dispersed across the tissue, that was more or
351 less absent from the tissues of mice by the 6th week of treatment.

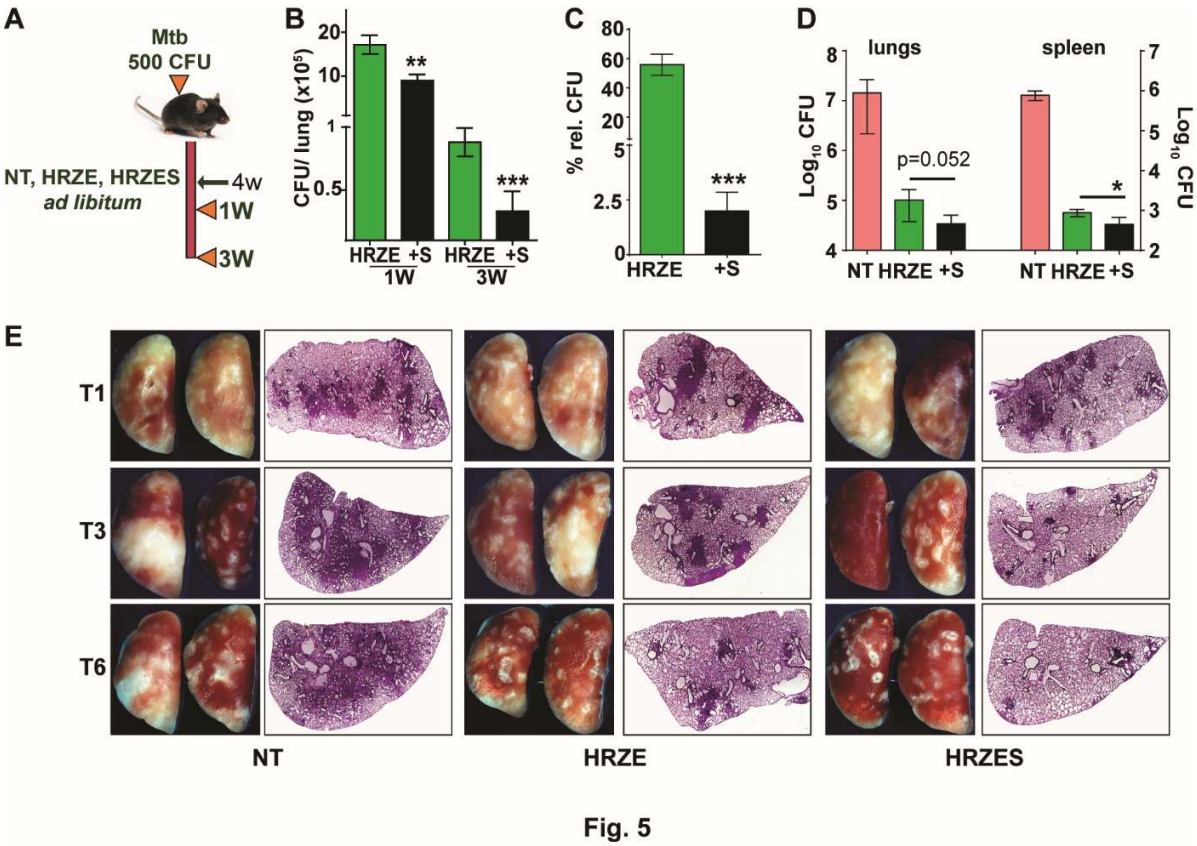

Fig. 5

Fig. 5: SRT initiates early bacterial clearance and dissemination in mice.

A) Schematic of Mtb infection and drug treatment in C57BL6/ BalbC mice infected with Mtb and the treated with HRZE or HRZES (HRC1- 1X: H-100μg/ml, R-40μg/ml, Z-150μg/ml, E-100μg/ml, SRT 10μg/ml) treatment. Lung CFU post 1 and 3 weeks (B) and spleen CFU 3 week after (C- relative to untreated animals) in Mtb infected C57BL6 mice of antibiotic and SRT administration. D) Lung and spleen CFUs of Balb/c mice after 3 weeks of treatment, E) Gross lung morphology and H&E staining after treatment for the indicated number of weeks. (unpaired t-test,*p<0.05, ***p<0.001).

We then tested the efficacy of the adjunct therapy against a drug tolerant Mtb strain in cellular and murine models of infection. Previously, we had demonstrated that N73- a clinical Mtb strain belonging to the L1 ancient lineage showed increasing tolerance to INH and Rifampicin as opposed to the modern L3 and L4 lineage strains by virtue of expressing the complete MmpL6 operon [61]. In THP1 cells infected with Mtb, HR at a concentration of 0.1X supported stasis and failed to decrease of bacterial numbers (Fig. 6A). The combination of HR with SRT, significantly

366 controlled infection and reduced bacterial numbers by ~10-15 folds by 3 days and ~100 folds by
367 day5. The pattern of significantly greater bacterial control was also observed in primary human
368 macrophages; again, a combination of HR and SRT reduced intracellular bacterial numbers by
369 5-50 folds in comparison to the drugs alone (Fig. 6B).
370 With a strong indication of SRT's ability to boost the efficacy of frontline TB drugs against
371 tolerant Mtb, we tested its *in vivo* activity in the acute model of C3HeB/ FeJ mouse infection in
372 combination with 0.1X HR (Fig. 6C). As expected, the drugs were not efficient in controlling
373 infection induced lesions in the lungs reducing bacterial numbers by 5 folds (Fig. 6D). However,
374 lungs of HRS treated animals harbored ~5-8 folds lower bacterial numbers than HR treated
375 animals. The effect of the combination was again better in controlling bacterial numbers in
376 spleens of treated mice wherein HR did not reduce CFU in contrast to the 6-8 fold lower
377 bacterial numbers with the combination.

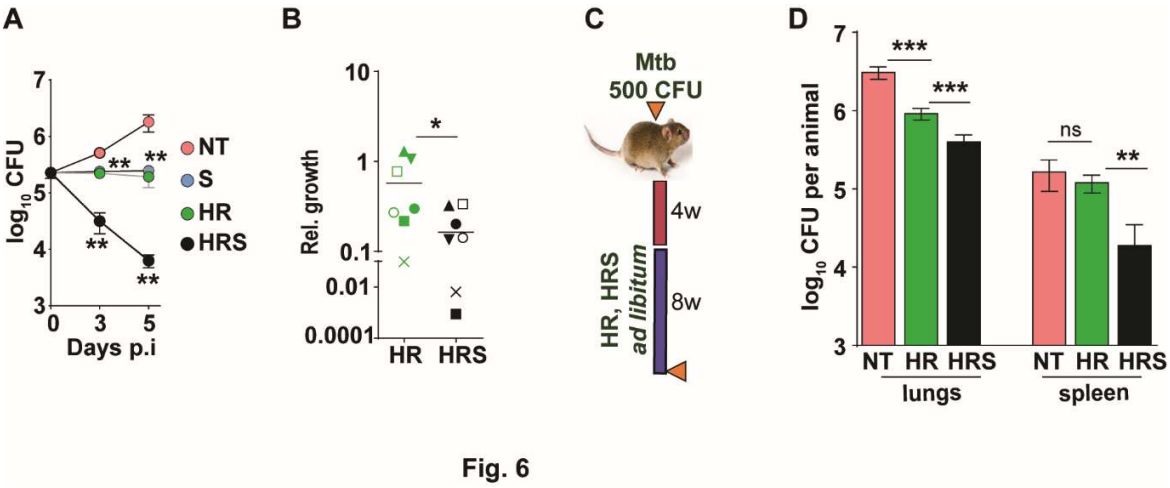

Fig. 6

378

379 **Fig. 6: Addition of SRT helps better control of drug tolerant Mtb in vivo**.
380 A) Intracellular bacterial growth in THP1 macrophages infected with HR tolerant Mtb strain at a MOI
381 of 5 for 6h. Following this, cells were left untreated (NT) or treated with SRT, HR or HRS for 3 or 5
382 days. Bacterial numbers were enumerated and is represented as average $\log_{10}$ CFU ± SEM from
383 two independent experiments with triplicate wells each. B) Bacterial numbers at day 3 of primary
384 monocyte derived macrophages (M1) from 7 independent donors. After 6h of infection, the

macrophages were treated with HR and HRS for 5 days. The ratio of intracellular bacterial numbers in HR or HRS groups with respect to untreated samples is represented as relative growth with median values indicated by the horizontal line. C) Schematic of Mtb infection and drug treatment in C3HeB/FeJ mice infected with Mtb for 4 weeks followed by treatment with 0.1X HR alone or with SRT (HRS) or 8 weeks. Bacterial numbers (CFU) in lungs and spleen (D) at the end of the experiment are shown as mean ± sd (unpaired t-test, ns=not significant, **p<0.01, ***p<0.001).

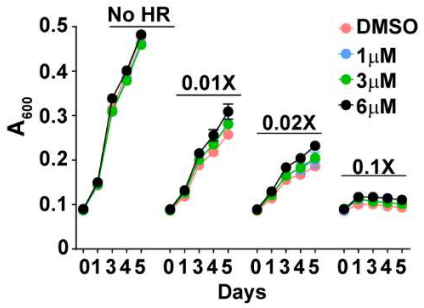

Fig. S1

Fig. S1: SRT does not augment the antibiotic efficacy of antibiotics *in vitro*.

In vitro growth of Mtb in 7H9 media containing different concentrations of SRT. Mtb cultures were either left untreated or in the presence of different SRT and HR concentrations for 5 days at 37°C. The growth was regularly monitored by measuring the optical density of culture and is represented as mean ± SD of triplicate assay wells of a representative experiment.

DISCUSSION

Despite consistent efforts in identifying novel pathogen targeted interventions and streamlined pharmaceutical drug development control processes, fewer drugs have been accepted for clinical use in TB over the last 40 years [62]. Repositioning existing drugs with established safety in humans is one of the quickest modes of developing effective control of infections that reduce the timeframe of regimen development. The need for an effective, short and pathogen-sterilizing regimen to tackle the growing problem of Mtb drug resistance and dormant bacterial populations has intensified efforts towards the development of host targeted therapies [63-66].

We and several other groups have identified type I IFN as an early response of host macrophages to infection with Mtb strains [46,47,53,67]. With recent evidences indicating this

412 response as a pathogen beneficial response, we hypothesized that attenuating this axis would
413 prove beneficial in controlling bacteria in macrophages. In line with this idea, we observed that
414 the previously reported TLR3 antagonist – sertraline (SRT) could effectively stunt Mtb induced
415 type I IFN response in macrophages and also inhibit bacterial growth in macrophages. More
416 importantly, the ability of SRT to potentiate antibiotic mediated killing was found to be driven by
417 perturbation of the inflammasome-Type I IFN axis. While several studies have identified an
418 important role for the host cell inflammasome in controlling bacterial infections, recent evidence
419 supports an antagonistic role for inflammasome activation on the type I IFN response of cells
420 [68,69]. A recent study has effectively demonstrated the importance of host inflammasome in
421 activating eicosanoids that control type I IFN thereby promoting bacterial clearance [70], [71].
422 Consistent with this observation, targeted therapy towards elevating PGE2 activity protected
423 mice from acute infection induced fatality [71]. Our results also highlight this cross- regulation
424 between two important innate response pathways with SRT boosting the macrophage pathogen
425 control program by repressing a pro- pathogenic and activating the host beneficial response. In
426 line with this observation, treatment with the inflammasome inhibitor Isoliquiritigenin reversed
427 the ability of SRT to enhance the effects of HR in macrophages. Previous evidences have
428 suggested a multi- faceted immune modulating property of SRT and other selective serotonin
429 reuptake inhibitors from enhancing the anti- inflammatory response [72] to enhancing NK and CD8
430 cell response[73] to inhibition of acid-sphingomyelinase [74], an essential component of the viral
431 trafficking into NPC1+ endosomes in cells. While our work points towards the type I IFN
432 antagonism mediated inflammasome activation as a key mechanism in providing synergy to anti
433 TB therapy, the exact molecular target of SRT in this process is an important future challenge.
434 SRT combination with front-line anti-TB drugs provided early bacterial control with improved
435 resolution of pathology and enhanced host survival, thereby being a strong candidate for host
436 directed therapy. SRT provides additional benefits as an adjunct modality. The pharmacological
437 properties of SRT has been well established with excellent PK PD, safety and tolerance for long

438 term usage in the human population [75-77]. Interestingly, two patients undergoing TB therapy with
439 INH given SRT as an anti- depressant, did not show any deleterious effects on long term use of
440 the combination, auguring well for safety in the human population[78,79]. In addition, these studies
441 combined with the enhanced protective capabilities of the combination therapy in pre-clinical
442 animal models (our data), rule out any possibility of negative drug-drug interactions between
443 SRT and ATT on prolonged usage. This long-term standard TB treatment is associated with
444 severe drug induced depression in patients. Recently depression was identified as an invisible
445 co-morbidity with TB with extensive synergistic action on the patient [80,81]. It is logical to expect
446 that SRT with its wide use as an anti- depressant in adults and children may be beneficial in
447 tackling this dual problem efficiently with a combination regimen of frontline TB drugs and SRT.
448 The combined properties of a SRT combination therapy of faster bacterial control, promoting
449 host survival and ability to target drug tolerant as well as dormant bacterial populations augurs
450 well for the highly constrained national/ global economy combating the TB pandemic.

451 **Material and Methods**

452 ***Bacterial Strains and Growth Conditions***—Mtb strains were cultured in Middlebrook 7H9
453 broth with 4% ADS or in 7H10/ 7H11 agar (BD Biosciences, USA) with 10% OADC (Himedia
454 laboratories, India).

455 **Reagents:** THP1 Dual Monocytes was obtained from InvivoGen (Toulouse, France). HiglutaXL
456 RPMI-1640 and 10% Fetal Bovine Serum (HIMEDIA laboratories, Mumbai, India), PMA
457 (Phorbol 12-Mysristate 13-acetate- P8139, Sigma Aldrich, USA), BX795 (tlrl-bx7, Invivogen)
458 were used for culture of cells. The following reagents were procured from Sigma Aldrich, USA:
459 Vit C (L- ascorbic acid, A5960), oleic acid albumin (O3008), Isoniazid (I3377), Pyrazinamide
460 carboxamide (P7136), Ethambutol dihydrochloride (E4630) and Sertraline hydrochloride
461 (S6319). Rifampicin (CMS1889, HIMEDIA laboratories, Mumbai, India) and commercially
462 available SRT (Daxid, Pfizer Ltd, India) was used for mouse studies.

463 **Macrophage infection:** THP1 Dual reporter monocytes were grown in HiglutaXL RPMI-1640
464 containing 10% FBS and differentiated to macrophages with 100nM PMA for 24h. Following a
465 period of rest for 48h, cells were infected with single cells suspensions (SCS) of Mtb at a MOI of
466 5 for 6h. For analyzing the Interferon (IRF pathway) activation levels, supernatants from Mtb
467 infected THP1 Dual macrophages were assayed for stimulation by measuring luminescence as
468 per manufacturer's recommendations.

469 **Monocyte derived macrophage culture**: PBMCs were isolated from fresh blood obtained from
470 healthy donors in accordance with Institutional human ethics committee approval (Ref no: CSIR-
471 IGIB/IHEC/2017-18 Dt. 08.02.2018). Briefly, 15-20 ml blood was collected in EDTA containing
472 tubes and layered onto HiSep (HIMEDIA laboratories, Mumbai, India) and used for isolation of
473 PBMCs according to the recommended protocols. Post RBC lysis, cells were seeded at a
474 density of $3x10^5$cells/ well and differentiated into monocyte derived macrophages with 50ng/ml
475 GMCSF for 7 days and then used for infection with Mtb.

476 **Analysis of response parameter**s: For analysis of different parameters of cellular response to
477 infection, qRTPCR based gene expression analysis and cytokine ELISA in culture supernatants
478 were performed according to manufacturer's recommendations.

479 **Analysis of gene expression by qRTPCR**

480 Total RNA was isolated from macrophages suspended in Trizol by using the recommended
481 protocol. cDNA was prepared from 1 µg of RNA by using the Verso cDNA synthesis kit and was
482 used at a concentration of 10ng for expression analysis by using the DyNAmo Flash SYBR
483 Green qPCR Kit (Thermo Fisher Scientific Inc., USA).

484 **Analysis of cytokine secretion by ELISA:**

485 Culture supernatants at different time intervals post infection/ treatment were filtered through a
486 0.2µ filter and subjected to ELISA by using the eBioscience (Thermo Fisher Scientific Inc. USA)
487 ELISA kit as per recommended protocols.

488 **Bacterial survival in macrophages**: For determining intra cellular survival of Mtb strains
489 macrophages were seeded in 48well plates and infected with Mtb at MOI 5 for 6 hours. SRT
490 and lyophilised BX795 were resuspended in DMSO and used at final concentration of 20 and
491 10μM, respectively. Frontline anti mycobacterial drugs were resuspended in DMSO as well and
492 used at different concentrations – C1, C2, C3: (C1- INH-200ng/ml, Rifampicin-1000ng/ml, -C2
493 and C3 :10- and 25-fold dilutions of C1). Macrophages were then treated with antibiotics INH,
494 and Rif with either BX795 or SRT or alone at specific concentrations. At specific days post
495 infection macrophages were lysed with water containing 0.05% of tween80. Dilutions of the
496 intracellular bacterial numbers were made in PBS with 0.05% of tween80 and plated on 7H10
497 agar plates. The effect of SRT on bacterial survival was tested in the VitC induced dormancy
498 model of macrophages as described earlier[55]. Macrophages were treated with 2mM Vit C for
499 24h and then treated with 0.1XHR for a further 3 days. For testing in lipid rich macrophages,
500 THP1 Dual monocytes were treated with oleic acid at 200μM concentration after PMA
501 differentiation for 2 days. These lipid rich macrophages were then infected with Mtb and treated
502 with antibiotics and SRT and checked for bacterial survival at day 5 post infection.

503 **Mouse infection and antibiotic treatment**: (6-10 weeks old) C3HeB/FeJ/ C57BL6/ Balbc
504 animals were infected with Mtb clinical isolate at 500 CFU per animal through aerosol route.
505 Two weeks post infection animals were started on antibiotics H (100mg/l), R (40mg/l) [82], Z
506 (150mg/l), E (100mg/l)[83] and SRT (10mg/l, human equivalent dose of 3.3 mg/kg/day), as
507 required treatment by giving all of the drugs *ad libitum* in their drinking water for 7 weeks which
508 was changed twice every week. For survival, animals were monitored regularly and euthanized
509 at a pre-determined end point according to the Institutional animal ethics approval. For
510 estimating tissue bacterial burdens, lungs and spleen of infected animals were collected in
511 sterile saline, subjected to homogenization and used for serial dilution CFU plating on 7H11
512 agar plates containing OADC as supplement. Colonies were counted after incubation of the
513 plates at 37°C for 3-5 weeks and recorded as CFU/tissue.

514 All statistical analysis was performed by using student's t-test for significance, P values of <
515 0.05 was considered significant.

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526

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528
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